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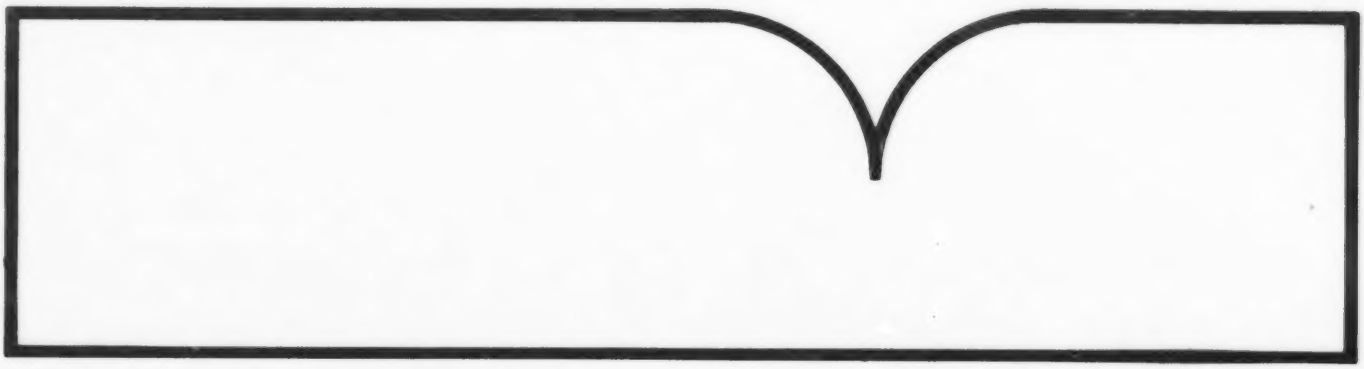

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Determination of Pentachlorophenol in Marine Biota and Sea Water by Gas–Liquid Chromatography and High-Pressure Liquid Chromatography

Linda F. Faas* and James C. Moore

A method is described for measuring pentachlorophenol (PCP) in samples from the estuarine environment. Gas–liquid chromatography (GLC) is used to determine PCP residues in tissues as low as 0.01 ppm by formation of the ethyl diazohydrocarbon derivative, followed by Florisil cleanup. Application of the method to exposed organisms indicates that PCP accumulates in mullet (*Mugil cephalus*), grass shrimp (*Palaemonetes pugio*), and eastern oysters (*Crassostrea virginica*). Sea water concentrations as low as 0.002 ppb may be detected by formation of the amyl diazohydrocarbon derivative. Formation of the amyl derivatives of PCP and several related compounds gives GLC separation not possible with the methyl or ethyl derivatives. Parameters are outlined for high-pressure liquid chromatography (LC) determination of the free phenol without cleanup. Ultraviolet detection limits for PCP by LC are 5.0 ppm in tissues and 2.0 ppb in seawater.

Pentachlorophenol (PCP) is a herbicide, fungicide/bactericide, and insecticide that, together with its salts, has a broad spectrum of industrial, agricultural, and domestic applications (Benvenue and Beckman, 1967). U.S. production of PCP in 1977 was expected to be 80 million pounds (Cirelli, 1978), and annual Canadian usage was estimated to be 20 million pounds (Hoos, 1978).

PCP has been found in drinking water (Abrams et al., 1975), rivers, lakes, and streams (Buhler et al., 1973; Fountaine et al., 1976; Pierce and Victor, 1978; Rudling, 1970), sewage effluents (Abrams et al., 1975; Buhler et al., 1973), aquatic biota (Pierce and Victor, 1978; Rudling, 1970; Zitko et al., 1974), and even in man (Barthel et al., 1969; Rivers, 1972).

The toxicity of PCP to aquatic organisms (Adelman et al., 1976; Benvenue and Beckman, 1967; Schimmel et al., 1978) and its effects on settling communities (Tagatz et

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KEYWORDS: *Water analysis, *Phenol/pentachloro, *Water pollution detection.

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al., 1977) have been investigated. Also, PCP contamination has been implicated in several fish kills (Pierce and Victor, 1978; Schimmel et al., 1978).

These data indicate that PCP poses a potential threat to the estuarine environment. Therefore, a sensitive reliable method is required for routine analysis of PCP in marine biota and seawater. A variety of analytical methods for analysis of PCP have been reported (Barthel et al., 1969; Benvenue and Beckman, 1967; Buhler et al., 1973; Fountaine et al., 1975; Pierce and Victor, 1978; Rivers, 1972; Rudling, 1970) but do not describe the determination of PCP in marine biota and sea water.

This paper describes the extraction of PCP from sea water and fish (*Mugil cephalus*), shrimp (*Palaemonetes pugio*), and oyster (*Crassostrea virginica*) tissues by formation of the ethyl and amyl diazohydrocarbon derivatives for GLC analysis, with Florisil cleanup for tissue samples. A technique for high-pressure liquid chromatography (LC) determination of PCP in tissues and sea water without derivatization or cleanup is outlined. Tables of retention times of the amyl derivatives illustrate the GLC separation of PCP from several related compounds.

EXPERIMENTAL SECTION

Apparatus. We employed Varian Models 1400 and 2100 gas chromatographs equipped with ^3H electron-capture detectors and 182 cm × 2 mm i.d. glass columns packed with 2% SP2100 on 100/120 mesh Supelcoport, 0.75% SP2250:0.97% SP2401 on 100–120 mesh Supelcoport or 5% QF-1 on 80–100 mesh Gas-Chrom Q. Operating parameters were: oven temperature, 180–190 °C; injector temperature, 210 °C; detector temperature, 200 °C; nitrogen carrier gas, 25 mL/min. Identity of the PCP ethyl and amyl derivatives was confirmed by gas chromatography–mass spectrometry (GC–MS), using a Finnigan Model 1015 mass spectrometer. The Waters LC system consisted of a Model U6-K injector, Model 440 UV absorbance detector operated at 254 nm and 0.01 aufs using a Model 6000A pump. A Waters μBondapak CN column with dimensions of 30 cm × 3.9 mm i.d. was used isocratically with 2.5% (v/v) isopropyl alcohol in isooctane at 2.0 mL/min. Initially, it was difficult to obtain reproducible peaks for PCP with LC. After repeated injections, however, conditions stabilized. Therefore, column conditioning may be necessary to achieve the LC results reported here. The tissue blender was a Willems Polytron Model 10-ST (Brinkman Instruments, Westbury, NY).

Reagents. All solvents were Nanograde (Burdick and Jackson Laboratories, Inc.). The *N*-nitrosoguanidine precursors used to generate diazohydrocarbons were obtained from Aldrich Chemical Company. (CAUTION: Extreme care must be exercised in handling *N*-nitrosoguanidine precursors that are potent mutagens, skin irritants, and carcinogen suspects.) The reagent grade anhydrous sodium sulfate was obtained from Baker Chemical Co. Florisil, PR Grade, 60–100 mesh (Floridin Company) was activated overnight at 130 °C before use.

Standards. Pentachlorophenol (PCP), 2,4,5-trichlorophenol, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methoxy-3,6-dichlorobenzoic acid (dicamba), and 2-(2,4,5-trichlorophenoxy)propionic acid (silvex) standards were obtained from the Pesticide Reference Standard Section, U.S. Environmental Protection Agency, Washington, DC. Tetrachlorohydroquinone, *p*-nitrophenol, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol were obtained from Chem Service, Inc., West Chester, PA.

Primary standards were prepared by diluting 100 mg to 100 mL with benzene. Working standards for GLC were prepared by diluting the primary standard solutions with petroleum ether. The PCP fortification standard was prepared by diluting the primary standard to 10 ng/μL with acetone. Working standards for LC were prepared in 2.5% (v/v) isopropyl alcohol in isooctane. All standards were stored in amber bottles closed with Teflon-lined screw caps.

Table I. Recoveries of PCP from Fortified Oyster, Shrimp, and Fish Tissues[a] and from Sea Water

	fortification level, ppm	derivative formed	no. of samples	percentage recov. $\bar{X} \pm SD$
fish (edible tissue)	0.05	ethyl	5	90.6 ± 13.3
oyster	0.05	ethyl	4	91.5 ± 3.8
shrimp (whole body)	0.05	ethyl	5	91.8 ± 6.1
fish (whole body)	15.0[b]		4	95.0 ± 6.5
	5.0[b]		15	89.4 ± 5.7
	1.0[c]	ethyl	5	95.8 ± 3.1
	0.01	amyl	5	97.2 ± 7.8

[a] Tissue samples were 3.0–10.6 g. [b] Determined by LC as the free phenol. [c] Extracted from 100 mL of sea water with 2–50-mL portions of 1:1 (v/v) diethyl ether/petroleum ether.

Procedures. One to eight grams of tissues were weighed into 150 × 25 mm o.d. screw-cap test tubes and extracted four times with 5-mL portions of acetonitrile for 30 s using a Willems Polytron tissue homogenizer. The test tube and contents were centrifuged after each extraction, and the acetonitrile was transferred to a 120-mL oil sample bottle. Seventy milliliters of 2% (w/v) aqueous sodium sulfate was added to the combined extracts. The pH was adjusted to 2–3 with 6 N HCl. (Aqueous sodium sulfate and 6 N HCl were prepared with distilled water that had been acidified and extracted with hexane to remove interfering coextractives.) This mixture was extracted with one 10-mL portion and then with two 5-mL portions of hexane. The bottle, sealed with a Teflon-lined screw cap, was shaken 1 min, the solvent phases were allowed to separate, and the upper hexane layer was pipetted into a 25-mL concentrator tube. The combined extracts were then concentrated to 0.5 mL by evaporation under a gentle stream of nitrogen while in a water bath maintained at a temperature of 40–50 °C. The ethyl derivative was formed by adding 10–12 drops of diazoethane, as described by Stanley (1966). Fresh diazohydrocarbons were prepared weekly and stored at −4 °C. (CAUTION: Gloves and a high-draft hood must be used in handling diazohydrocarbons, which are toxic and potentially explosive carcinogens.) This mixture was allowed to stand at room temperature for 20 min, and the excess diazoethane was evaporated with a gentle stream of nitrogen. A 9-mm Chromaflex column (Kontes Glass Company, Vineland, NJ) was filled with 3.0 g of Florisil topped with 2.0 g of anhydrous sodium sulfate and washed with 10 mL of hexane. The concentrate was quantitatively transferred to the column with three 0.5-mL portions of hexane and the ethyl derivative was eluted with 20 mL of 5% (v/v) diethyl ether in hexane. The volume was adjusted to the appropriate concentration for analysis by GLC.

For water samples, 1 L of sea water was extracted twice with 100-mL portions of 1:1 (v/v) diethyl ether/petroleum ether in a 2-L separatory funnel. The extract, collected in a Kuderna-Danish concentrator, was evaporated to 5 mL on a steam table with a Snyder column. The concentrator tube was transferred to a nitrogen evaporator; the extract was concentrated to 0.5 mL, derivatized with

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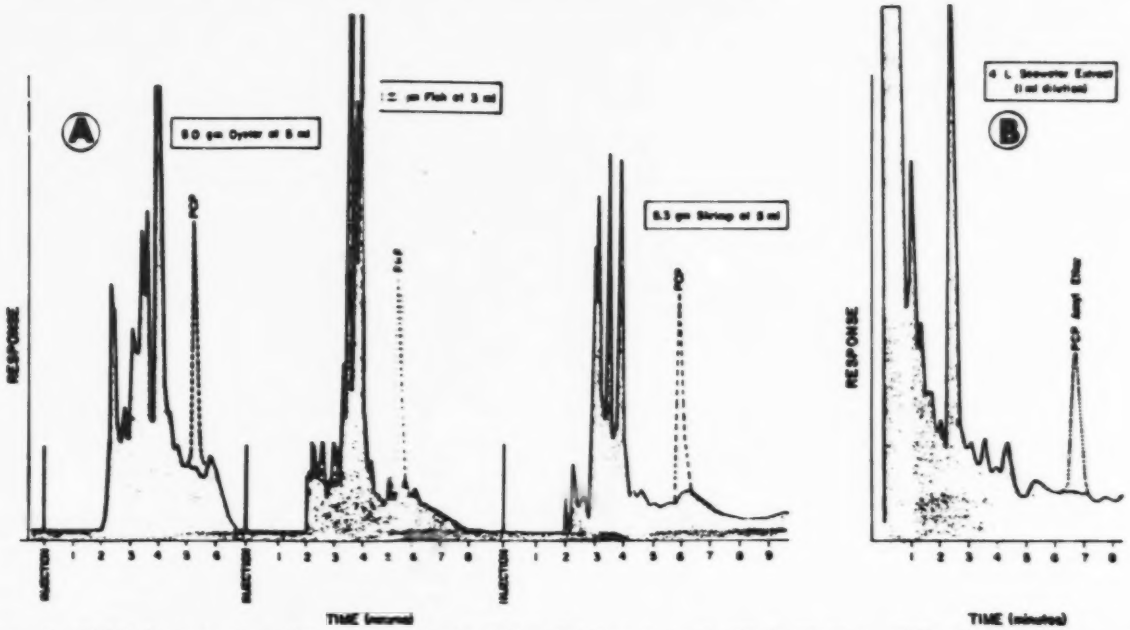

Figure 1. (A) LC chromatograms of the extracts of oyster, fish, and shrimp tissues fortified with 15 ppm PCP. Shaded area represents chromatogram of blank tissue extracts. Fish tissue extract was cleaned with a basic methylene chloride extraction; oyster and shrimp tissues were not. Amount injected was 15 μL. B: GLC chromatogram of combined four 1-L seawater extracts. Shaded area represents combined blank extracts and PCP concentration is 0.002 ppb. Amount injected was 5 μL.

Table II. Measured Residues of PCP in Fish, Shrimp, and Oysters Exposed to Several Measured Concentrations of PCP in Flowing Sea Water for 96 h

species	concn in water, ppb	tissue residues, ppm
fish (*Mugil cephalus*)	46.0	0.29
	85.0	6.7
	157.0	8.8
shrimp (*Palaemonetes pugio*)	32.0	0.050
	54.0	0.10
	76.0	0.23
	249.0	0.43
oyster (*Crassostrea virginica*)	2.9	0.18
	26.0	0.86

diazoethane for GLC analysis, and analyzed without Florisil cleanup.

Interference with the PCP ethyl derivative was often observed for PCP determinations in sea water at concentrations lower than 0.01 ppb. To reduce this background, sea water was extracted at pH 9 with methylene chloride before extracting PCP at pH 2–3. Also, vent-peak tailing and interference from coextractives or impurities in the diazohydrocarbons were decreased by washing the extract with 5 mL of 20% (v/v) distilled water in methanol (Thompson, 1974). All glassware was washed with 1 N KOH, distilled water, and Nanograde acetone (Thompson, 1974). Formation of the amyl derivative increased retention time sufficiently to separate PCP from early eluting peaks that were observed. The diazopentane was prepared by the method of Shafik et al. (1973), and derivatization of PCP was carried out in the same manner as formation of the ethyl derivative.

For analysis by LC, sea water and tissues were extracted as outlined above, without derivatization or Florisil cleanup. However, for tissue analyses, a basic methylene chloride extraction was sometimes necessary to reduce background interference (primarily in fish extracts). The aqueous sodium sulfate solution was adjusted to pH 9 and the solution extracted once with 10 mL of methylene chloride. The methylene chloride was discarded, the pH of the remainder was adjusted to 2–3, and the solution was extracted with hexane as above.

A known amount of the PCP fortification standard was added to sea water and tissue samples just prior to analyses to determine recoveries. The standard for comparison was

Table III. Retention Times of Ethyl and Amyl Derivatives of Several Phenols and Acids Relative to Aldrin on Three Different GLC Columns

	2% SP 2100		0.75% SP2250:0.97% SP 2401		5% QF-1	
compound	ethyl	amyl	ethyl	amyl	ethyl	amyl
2,4,6-trichlorophenol	0.14	0.40	0.13	0.34	0.17	0.40
p-nitrophenol	0.17	0.52	0.25	0.65	0.55	1.38
2,4,5-trichlorophenol	0.20	0.54	0.20	0.51	0.28	0.64
dicamba	0.27	0.72	0.32	0.85	0.51	1.19
2,3,4,6-tetrachlorophenol	0.28	0.79	0.27	0.73	0.34	0.79
2,4-D	0.38	0.98	0.48	1.24	0.79	1.82
silvex	0.57	1.41	0.63	1.58	0.96	2.06
pentachlorophenol	0.55	1.54	0.56	1.52	0.64	1.47
2,4,5-T	0.62	1.64	0.80	2.11	1.20	2.70
tetrachlorohydroquinone	0.65	5.37	0.68	5.30	0.83	4.45
aldrin	1.00	1.00	1.00	1.00	1.00	1.00

prepared from the fortification standard.

RESULTS AND DISCUSSION

The GLC chromatograms of fish, shrimp, and oysters that had been exposed to or fortified with PCP were clean and showed no interfering peaks. The lower limit of detection for PCP in tissues by the GLC method with ethyl derivatization and Florisil cleanup was 0.01 ppm.

LC offers a rapid method for determination of PCP residues in tissues and sea water without derivatization or Florisil cleanup. Concentrations of PCP above 2.0 ppb in sea water and 5.0 ppm in tissues can be quickly determined by this method. LC chromatograms of PCP recovered from fortified oyster, shrimp, and fish tissues are shown in Figure 1A.

Table I lists the average percentage recovery of PCP from fortified fish, shrimp, and oyster tissues and sea water by the above extraction procedures. Residues measured in tissues of exposed animals are shown in Table II. These residues were determined by GLC after formation of the ethyl derivative and Florisil cleanup.

Formation of the amyl derivative can be used to separate PCP from impurities, coextractives and several related compounds. Figure 1B illustrates the composite extract of 4 L of sea water. By using the amyl derivative and techniques to determine concentrations of PCP less than 0.01 ppb in sea water, PCP can be resolved from background at 0.002 ppb.

From the retention times of the ethyl and amyl derivatives of PCP and several related compounds listed in Table III, it can be seen that the amyl derivatives increase retention times sufficiently to give GLC separation not possible with the ethyl derivatives. Although no one column provides complete resolution, a combination of any two columns will allow separation of all ten compounds. The amyl derivative can be used for tissue as well as sea water samples should PCP be present in combination with any of these compounds.

Our method, successfully tested in the estuarine environment, offers: routine application, minimum cleanup, elimination of interference with coextractives and several related compounds observed with other derivatives, and improved sensitivity.

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